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Specific interaction of ivermectin with retinol-binding protein from filarial parasites

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Specific cellular binding proteins for retinol and retinoic acid from mammalian and avian species may mediate the action of retinoids in the control of epithelial differentiation, growth and tumorigenesis. Parasite retinol-binding protein (PRBP) and parasite retinoic acid-binding protein (PRABP) isolated and characterized from parasitic worms of the family Filarioidea might be involved in some possible action of vitamin A compounds in these parasites. Ivermectin, a potent and widely used anti-parasitic drug, competes efficiently with retinol for retinol-binding sites on PRBP, but not for the host-tissue retinol-binding-protein sites. The drug has no affinity for retinoic acid-binding proteins from either parasite or host tissues. Binding studies using radiolabelled ivermectin and retinol reveal that ivermectin has a higher affinity than retinol for PRBP. A correlation exists between the binding affinities of ivermectin analogues and their anti-parasitic activities. A binding-protein-mediated interrelationship may exist between the actions of retinol and ivermectin in the parasites, but not in the host tissues.

INTRODUCTION

The essential nature of vitamin A (retinol) for maintenance of epithelial tissues was first brought to light by the elegant histopathological studies by Wolbach & Howe (1925). Nutritional effects of vitamin A compounds (retinoids) are multifarious (Wolf, 1984). Various attempts to determine the molecular mechanism of action of retinoids in the control of epithelial differentiation, growth, reproduction and tumorigenesis have centred on retinoid-binding proteins (Sani & Banerjee, 1983; Chytil & Ong, 1984; Goodman, 1980). It was suggested by Bashor *et al.* (1973) and by Sani & Hill (1974) that the action of retinol and retinoic acid may be mediated by their cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP). These binding proteins may mediate the biological processes that involve migration of the retinoids from the cell-surface membrane through the cytoplasm to the nucleus, where they exert specific effects upon gene expression (Sani & Banerjee, 1983; Chytil & Ong, 1984).

The role of retinoids in parasites is a relatively unexplored area, although the occurrence and uptake of retinol, as well as its formation from β-carotene, have been reported (Comley & Jaffe, 1983). Growth of some developmental stages of helminth parasites may depend on host levels of retinol (Mahalanabis *et al.*, 1976). Sturchler *et al.* (1983) showed that the retinol concentration in adult *Onchocerca volvulus* was 8 times higher than that of the surrounding host tissues. Although the exact function of retinoids in parasites is not clear, it may be assumed, by analogy to host tissues, that they control certain vital biological functions of the parasites. In our efforts to study the interactions of retinoids with parasitic components, we discovered and partially characterized specific parasite retinol-binding protein (PRBP) and parasite retinoic acid-binding protein (PRABP) from several filarial parasites (Sani & Comley, 1985; Sani *et al.*, 1985). Some of the physicochemical characteristics of the parasite binding proteins are distinct from those of the host-tissue binding proteins (Sani *et al.*, 1985). In particular, the protein–ligand interactions in host tissues were sensitive to mercurials, whereas they were insensitive in parasitic tissues.

Since retinoids may be involved in the control of vital biological functions of the parasites, and since their specific binding proteins, PRBP and PRABP, may be important mediators of such functions, it is possible that the physicochemical differences that were observed between the host and parasitic binding proteins could be usefully exploited in the selective inhibition of parasitic growth. With these considerations, we started examining the binding affinities of known anti-parasitic agents for parasite retinoid-binding proteins, PRBP and PRABP, as distinct from their binding affinities for CRBP and CRABP, their counterparts in the host. Avermectins are a recently discovered family of natural compounds (Campbell *et al.*, 1983; Campbell & Benz, 1984; Fisher & Mrozik, 1984; Koch, 1984) produced by *Streptomyces avermitilis* which, at extremely low dosage, are active against nematode and anthropod parasites. The efficacy of these drugs against the skin-dwelling microfilariae of *Onchocerca* species in horses, cattle and humans makes avermectins a promising family of anti-parasitic drugs. We now report that the most potent drug, ivermectin (22,23-dihydroavermectin B_{1a}; Campbell *et al.*, 1983; Fisher & Mrozik, 1984), completely inhibited the binding of [^{3}H]retinol to PRBP; the drug showed virtually no inhibition of retinol binding to CRBP from the host tissues.

EXPERIMENTAL PROCEDURES

Studies on [^{3}H]retinol binding to PRBP and its inhibition by avermectins were accomplished by using

Abbreviations used: PRBP, parasite retinol-binding protein; PRABP, parasite retinoic acid-binding protein; CRBP and CRABP, cellular retinol- and retinoic acid-binding protein respectively from mammalian and avian species; GABA, γ-aminobutyric acid.

sucrose-density-gradient-sedimentation techniques (Sani *et al.*, 1984). The soluble extracts (2.0 mg of protein in 0.3 ml of 50 mM-Tris/HCl, pH 7.2) from *O. gibsoni*, *O. volvulus*, *Dipetalonema viteae*, *Brugia pahangi*, *Dirofilaria immitis*, *Schistosoma mansoni*, materials from the walls of *O. gibsoni* nodules (bovine origin), *O. volvulus* nodules (human origin), rat kidney, rat small intestine, rat liver, rat testes, rat eye and rat colon (Sani *et al.*, 1984, 1985) were incubated with 200 pmol of [15-^{3}H]retinol (1.66 Ci/mmol) or [22,23-^{3}H]ivermectin (10 Ci/mol) in the presence or absence of 200-fold molar excess of the test compounds, and the preparations were dialysed. The 2 S-binding-protein peaks were determined from the radioactivity profiles obtained after sedimentation of the above preparations through 5–20 %-(w/v)-sucrose density gradients at 180000 *g* for 18 h. In order to measure the relative binding affinity of the test compounds for PRBP, the inhibition of [^{3}H]retinol-binding caused by 200-fold molar excess of unlabelled retinol is regarded as 100 % inhibition (Sani *et al.*, 1978, 1984). The inhibition caused by a 200-fold molar excess of the test compounds is expressed as inhibition relative to unlabelled retinol.

RESULTS AND DISCUSSION

Fig. 1 illustrates the sucrose-gradient-sedimentation profiles of [^{3}H]retinol in the presence or absence of 200-fold molar excess of retinol or ivermectin after incubation with soluble protein extracts from *O. volvulus* and rat kidney. Ivermectin showed 100 % inhibition of [^{3}H]-retinol binding to PRBP, whereas the drug was without any effect on the binding of [^{3}H]retinol to CRBP from rat kidney. As defined, retinol showed 100 % inhibition of binding of [^{3}H]retinol to either PRBP or CRBP. The results indicate that ivermectin clearly distinguishes between retinol-binding sites on the parasitic and the host-tissue binding proteins.

Ivermectin, like retinol, showed 100 % inhibition of [^{3}H]retinol binding to PRBP from *O. gibsoni*, *D. immitis* and *B. pahangi* (Table 1). However, unlike retinol, ivermectin failed to show any significant inhibition of retinol binding to CRBP from any of the rat tissues tested (Table 1). This observation may explain the selective action of ivermectin against filarial parasites. We previously reported the presence of specific 2 S binding proteins for both retinol and retinoic acid from *O. volvulus* nodules (human origin) and *O. gibsoni* nodules (bovine origin) (Sani *et al.*, 1985). These binding proteins demonstrated mercurial-sensitive protein–ligand interactions that are typical of host-tissue binding proteins. We have now observed that ivermectin produced no significant competitive binding affinity for the retinol-binding sites on these nodular binding proteins (Table 1). Sucrose-density-gradient patterns of [^{3}H]-retinol-soluble protein complexes from *Schistosoma mansoni* revealed a 5 S-radioactive-binding-protein peak instead of the 2 S-binding-protein peaks exhibited by the filarial parasites. Ivermectin, shown to be ineffective against schistosomes (Fisher & Mrozik, 1984; Koch, 1984), did not demonstrate affinity for the retinol-binding site on this 5S protein. Although ivermectin abolished [^{3}H]retinol binding in filarial parasites, the drug was virtually without any competition with [^{3}H]retinoic acid for the binding sites on PRABP or CRABP (results not shown). This indicates that ivermectin may not interfere with any possible parasitic functions displayed by the retinoic acid–PRABP complex.

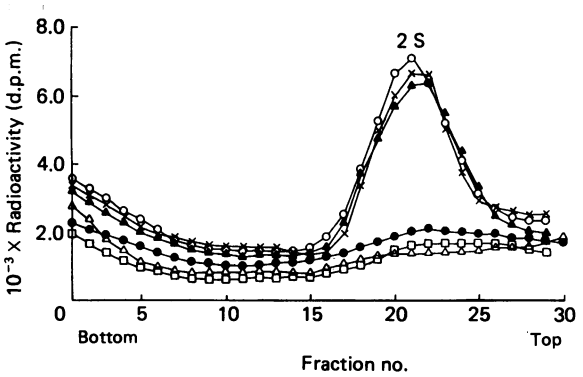

Fig. 1. Sucrose-density-gradient-sedimentation profiles of *O. volvulus* extract (1.5 mg of protein) and rat kidney cytosol (2.0 mg of protein) plus [^{3}H]retinol (200 pmol) in the presence or absence of 200-fold molar excess of retinol or ivermectin

○, *O. volvulus* + [^{3}H]retinol; ●, *O. volvulus* + [^{3}H]-retinol + retinol; △, *O. volvulus* + [^{3}H]retinol + ivermectin; ×, rat kidney + [^{3}H]retinol; □, rat kidney + [^{3}H]retinol + retinol; ▲, rat kidney + [^{3}H]retinol + ivermectin.

Table 1. Inhibition of binding of [^{3}H]retinol to retinol-binding proteins in (*a*) parasites and (*b*) tissues by ivermectin

(*a*) Parasite	Relative inhibition of binding of [^{3}H]-retinol to PRBP by ivermectin (%)
O. gibsoni	100
O. volvulus	100
D. immitis	100
B. pahangi	100
S. mansoni	0

(*b*) Tissue extract	Relative inhibition of binding of [^{3}H]-retinol to CRBP by ivermectin (%)
Rat kidney	0
Rat testes	15
Rat liver	10
Rat eye	10
Rat small intestine	15
Rat colon	0
O. volvulus-nodule-wall material	15
O. gibsoni-nodule-wall material	15

Although ivermectin inhibits the binding of [^{3}H]retinol to PRBP, we had no evidence that the retinol-binding sites were in fact occupied by ivermectin. Fig. 2(*a*) represents the sucrose-gradient-sedimentation pattern of *O. gibsoni* and rat kidney extracts after incubation with [^{3}H]ivermectin. *O. gibsoni* extracts display a 2 S radio-

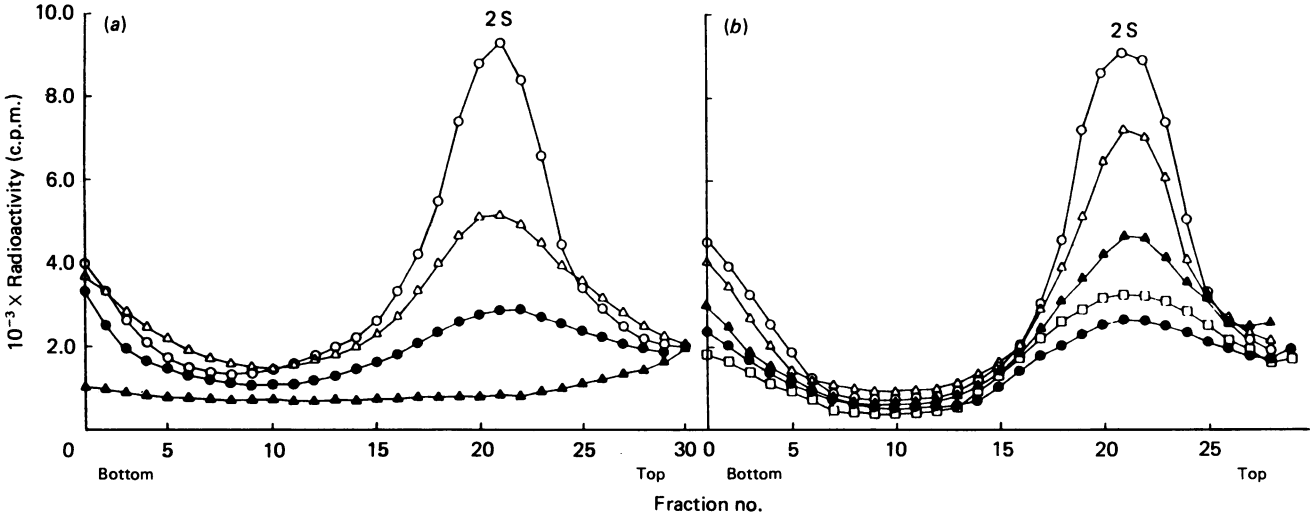

Fig. 2. Sedimentation patterns on sucrose gradients

(*a*) *O. gibsoni* extract (1.5 mg of protein) or rat kidney cytosol (2 mg of protein) plus [^{3}H]ivermectin (200 pmol) in the presence or absence of 200-fold molar excess of retinol or ivermectin. ○, *O. gibsoni*+[^{3}H]ivermectin; ●, *O. gibsoni*+[^{3}H]ivermectin+ivermectin; △, *O. gibsoni*+[^{3}H]ivermectin+retinol; ▲, rat kidney+[^{3}H]ivermectin. (*b*) *O. gibsoni* extract (1.5 mg of protein)+[^{3}H]ivermectin (control) with or without a 200-fold molar excess of the following unlabelled avermectins: ○, control; ●, ivermectin; □, 13-deoxy-22,23-dihydroavermectin B_{1a} aglycone; ▲, 22,23-dihydroavermectin B_{1a} aglycone; △, 5-oxoavermectin B_{1a}.

active peak attributable to ivermectin-binding protein. Competition with unlabelled ivermectin abolishes this peak almost completely, indicating the specific nature of binding. Competition with a similar molar excess of retinol, however, diminished the peak only to about 50%. This suggests that ivermectin has a higher affinity than retinol for the retinol-binding sites on PRBP and may explain why ivermectin displays anti-parasitic activity at extremely low concentrations. A significant observation was that rat kidney cytosol failed to express an [^{3}H]ivermectin-binding protein peak (Fig. 2*a*), indicating that ivermectin has no affinity for either CRBP or any other proteins in host tissues.

Several members of the avermectin family express different degrees of anti-parasitic activity (Campbell *et al.*, 1983; Campbell & Benz, 1984; Fisher & Mrozik, 1984; Koch, 1984). We wanted to verify whether such activities are related to their binding affinities for PRBP. Like ivermectin, 13-deoxy-22,23-dihydroavermectin B_{1a} aglycone is a highly active ivermectin analogue. Competition studies using these two avermectins against [^{3}H]-retinol for PRBP binding reveal that both avermectins compete almost similarly for the retinol-binding sites (Fig. 2*b*). 22,23-Dihydroavermectin B_{1a} aglycone, which shows moderate activity in parasites, does not compete as well as ivermectin does for the binding sites on PRBP. 5-Oxoavermectin B_{1a}, which is a weakly active analogue, is a poor competitor against [^{3}H]retinol for PRBP. Thus a correlation is evidenced between the anti-parasitic activity and binding affinity for PRBP among the four avermectins that were examined.

Our data show that (i) both retinol and ivermectin bind to the 2 S PRBP extracted from the microfilariae and (ii) there exists a cross-competition of the drug for retinol's binding site or for retinol for ivermectin's binding site. A similar cross-competition is absent in the host tissues. The competition between ivermectin and

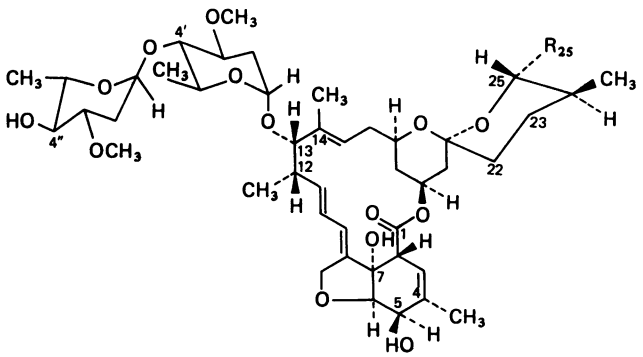

Fig. 3. Chemical structures of retinol and ivermectin

In the a-series of ivermectins R_{25} is $CH(CH_3)C_2H_5$; in the b-series, R_{25} is $CH(CH_3)_2$.

retinol via PRBP is intriguing, especially because they possess dissimilar chemical structures (Fig. 3). A common hydrophobic moiety in their structures may have recognized the binding site on the protein. Retinol is involved in the differentiation and maintenance of epithelial tissues, growth promotion, reproduction and vision. Since parasites apparently lack visual function, the retinol concentrated in them may be utilized in the control of the rest of the biological roles of vitamin A. We do not yet know whether ivermectin blocks one or all of those vital functions in the parasite, nor do we know

the efficacy of pharmacological levels of ivermectin to be operative by such a mechanism. It is known that, in susceptible parasites, ivermectin acts as a potent stimulant of GABA-mediated Cl^- conductance and interferes with GABA-mediated signal transmission from interneurons to excitatory motor neurons (Fisher & Mrozik, 1984). Although paralysis is the most evident effect, suppression of reproductive processes also has been reported (Fisher & Mrozik, 1984; Kass *et al.*, 1980). Although retinol has not been implied so far in GABA-mediated functions, it is involved in reproduction. At this stage in our understanding of the interrelationship between the actions of retinol and ivermectin, we may only conclude that their cross-competition could be important in the chemotherapy of filarial parasites.

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